JOURNAL OF BACTERIOLOGY, Mar. 1989, p. 1379–1385
0021-9193/89/031379-07$02.00/0

Vol. 171, No. 3

The *groES* and *groEL* Heat Shock Gene Products of *Escherichia coli* Are Essential for Bacterial Growth at All Temperatures

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Received 16 September 1988/Accepted 22 November 1988

The products of the *groES* and *groEL* genes of *Escherichia coli*, constituting the *groE* operon, are known to be required for growth at high temperature (42°C) and are members of the heat shock regulon. Using a genetic approach, we examined the requirement for these gene products for bacterial growth at low temperature (17 to 30°C). To do this, we constructed various *groES groEL* heterodiploid derivative strains. By inactivating one of the *groE* operons by a polar insertion, it was shown by bacteriophage P1 transduction that at least one of the *groE* genes was essential for growth at low temperature. Further P1 transduction experiments with strains that were heterodiploid for only one of the *groE* genes demonstrated that both *groE* gene products were required for growth at low temperature, which suggested a fundamental role for the *groE* proteins in *E. coli* growth and physiology.

The *groES* (*mopA*) and *groEL* (*mopB*) genes of *Escherichia coli* form an operon located at 94.2 min on the standard genetic map (2). They were first defined by mutations affecting the morphogenesis of several bacteriophages, including λ, T4, and T5 (see reference 6 for a review). Both of the *groE* gene products have been shown to be essential for bacteriophage λ head assembly (6–8, 33) and for bacteriophage T5 tail assembly (8, 40). In addition, the *groEL* gene product has been shown to be required for proper T4 head assembly (6, 7, 29, 32). Some alleles of both genes were subsequently shown to also result in thermosensitive bacterial growth at 42°C, affecting both DNA and RNA synthesis (*ts* mutants; 36). Both the bacterial temperature-sensitive phenotype and inability to propagate bacteriophage λ always contransduced, which demonstrated that the *groE* gene products are required for bacterial growth at least at high temperature (7). Although the exact role of these gene products in cell physiology remains to be determined, several of their properties are known at the physiological and molecular levels. The *groES* and *groEL* genes code for 10,368- and 57,259-M_r acidic polypeptides, respectively, found at high intracellular levels (about 2% of total cell proteins at 37°C; 12, 26, 33). Furthermore, as members of the heat shock regulon, the intracellular levels of their products increase with temperature through a positive transcriptional control exerted by the *rpoH* (σ^{32}) gene product (10).

The products of some of the heat shock genes are either totally indispensable (e.g., *rpoD*, which codes for the σ^{70} subunit of *E. coli* RNA polymerase; 26), dispensable (e.g., *lon*, which codes for an ATP-dependent protease [22, 26], or *lysU*, which codes for an alternate form of lysyl-tRNA synthetase [26, 34]), or conditionally dispensable; i.e., deletion mutants can be constructed at low temperature but grow poorly and rapidly accumulate extragenic suppressors [e.g., *dnaK* [27; B. Bukau, C. E. Donnelly, and G. C. Walker, *in* M. L. Pardue, J. Feramisco, and S. Lindquist, ed., Stress-Induced Proteins, in press] and *dnaJ* [S. Sell and C. Georgopoulos, unpublished data]). In this article, we present data demonstrating that, like the *rpoD* gene product, the *groE* gene products are essential for bacterial growth not only at high temperature (42°C) but also at low temperature (17°C).

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MATERIALS AND METHODS

Bacterial strains, bacteriophages and plasmids. The bacterial strains, bacteriophages, and plasmids used in this study are listed in Table 1. The key properties of the strains as well as some of the construction steps are also given in Table 1. The following abbreviations are used: Ts, temperature sensitivity; Tr, temperature resistance; bp, for base pairs; kbp, 1,000 bp of DNA; Tet^r and Tet^s, resistance and sensitivity, respectively, to tetracycline; Kan^r, resistance to kanamycin; Amp^r, resistance to ampicillin; and Ssm^r and Ssm^s, resistance and sensitivity, respectively, to streptomycin and spectinomycin.

Genetic procedures. Bacteria were routinely grown in LB medium (25). Bacteriophage P1 stocks were prepared according to Miller (25), as were the bacteriophage P1 transductions. Selection medium had the following composition (per liter): 15 g of Bacto-Agar (Difco Laboratories, Detroit, Mich.); 5.4 g of Na_2HPO_4; 3 g of KH_2PO_4; 0.5 g of NaCl; 1 g of NH_4Cl; 1.5 g of sodium citrate; 50 mg of thymine; 5 g of tryptone (Difco); 2.5 g of yeast extract (Difco); and 20 mg of tetracycline, 25 mg of kanamycin, or 60 mg of spectinomycin. After a 1-h incubation at 30°C to allow phenotype expression, each P1-infected culture was mixed with 4 ml of low-agar medium (i.e., selection medium without antibiotic and containing only 7.5 g of agar per liter) and poured over a selective plate.

Strain OF216 (Table 1) was expected to contain the *groES3*::Ω insertion in the 94.2-min region on the *E. coli* genetic map (2). This was verified by transducing it to Tet^r with a P1 lysate grown on strain CG712 (Table 1), which carries the *zjd-1*::Tn*10* insertion located at 93.7 min. As expected, about 60% of the Tet^r transductants had lost the Ssm^r phenotype. One of the Tet^r Ssm^r transductants from the same transduction was saved (strain OF246 in Table 1). The close linkage between the Tet^r and Ssm^r markers was verified before strain OF246 was used.

Manipulations involving bacteriophage λ derivatives

TABLE 1. Bacteria, bacteriophages, and plasmids used

Bacterial strain, phage, or plasmid	Relevant genotype[a] or phenotype	Source or reference
E. coli strains		
CB0129	(F^-) W1485 *leu thi thyA deoB* or *-C supE*42?	Our collection
CG712	(F^-) W3110 *galE relA groES30 zid-1*::Tn*10*	Our collection
OF197	CB0129(λ117)	This study
OF216	CB0129 *groES3*::Ω(λ117)	Infection of OF197 with λOFP29, selection of Ssm^r clones and screening for an imm^{λ}-sensitive clone
OF246	CB0129 *groES3*::Ω*zjd-1*::Tn*10*(λ117)	Transduction of OF197 to Tet^r with phage P1 grown on CG712
OF323	(F^-) CG39 *zbh-5*::Kan^r	Insertion of mini-Tn*10*-Kan^r (37) near *att*λ (90% cotransduction)
OF341	CB0129/λ; resistant to h^{λ}	This study
OF361	OF341(pJB8)	This study
OF362	OF341(pOF39)	This study
OF363	OF341(pS4)	This study
OF364	OF341(pOF58)	This study
OF377	CB0129 *gal*::Kan^r	Insertion of mini-Tn*10*-Kan^r (37) in the *gal* operon
Bacteriophages		
P1	P1L4	5
λ clear	λ imm^{λ} *c*I *b*2	Our collection
λSdal	λ imm^{λ} cI^+ Δ(*int xis* cIII)::8.1 kbp (*groES*$^+$ *groEL*$^+$) *Eco*RI fragment	5
λOFP29	λSdal *groES3*::Ω	An Ssm^r-transducing phage was selected after in vivo recombination of λSdal with plasmid pOF27 (described below)
λ117	λ imm^{21} cI^+::8.1 kbp (*groES*$^+$ *groEL*$^+$) *Eco*RI fragment	In vivo recombination between λ imm^{21} cI^+ and λ375 of Tilly et al. (33)
λ117 clear	λ imm^{21} *c*::8.1 kbp (*groES*$^+$ *groEL*$^+$) *Eco*RI fragment	Clear-plaque derivative (unmapped mutation of λ117)
Plasmids		
pLN44	Amp^r Tet^r *groES*$^+$ *groEL*$^+$	Cloning of the 8.1-kbp *Eco*RI fragment of λSdal into pBR325 (5)
pOF12	Amp^r Tet^r *groES*$^+$ *groEL*$^+$	Cloning of the 8.1-kbp *Eco*RI fragment of λ375 into pBR322 (5)
pOF13	Amp^r Tet^r *groES313 groEL*$^+$	Generation of a 2-bp deletion in *groES* by nuclease S1 treatment of *Sac*II-digested pOF12 (5)
pOF27	Amp^r Tet^r Ssm^r *groES3*::Ω*groEL*$^+$ ($GroEL^-$)[b]	Insertion of the *Sma*I-digested Ω fragment, carrying the Ssm^r gene (28) in the unique *Sac*II site of pLN44 located within the *groES* gene
pOF39	Amp^r *groES*$^+$ *groEL*$^+$	Tet^s derivative of pLN44 (5)
pOF58	Amp^r Tet^s *groES313 groEL*$^+$ ($GroEL^+$)[c]	Inactivation of the Tet^r gene of pOF13 by deletion of the 0.3-kbp *Hin*dIII fragment
pJB8	Amp^r	pBR322 derivative (13)
pS4	Amp^r *groES*$^+$	Cloning of a 0.95-kbp *Sau*3A fragment from pOF12 into pJB8 (5)

[a] Genotypes are given according to Bachmann (2).
[b] ($GroEL^-$) indicates that the *groEL*$^+$ gene, although wild type in sequence, is not expressed because of a polar effect of the Ω insertion in the first gene of the operon.
[c] ($GroEL^+$) indicates that, in spite of the frameshift mutation in *groES*, the *groEL*$^+$ gene is still expressed (5).

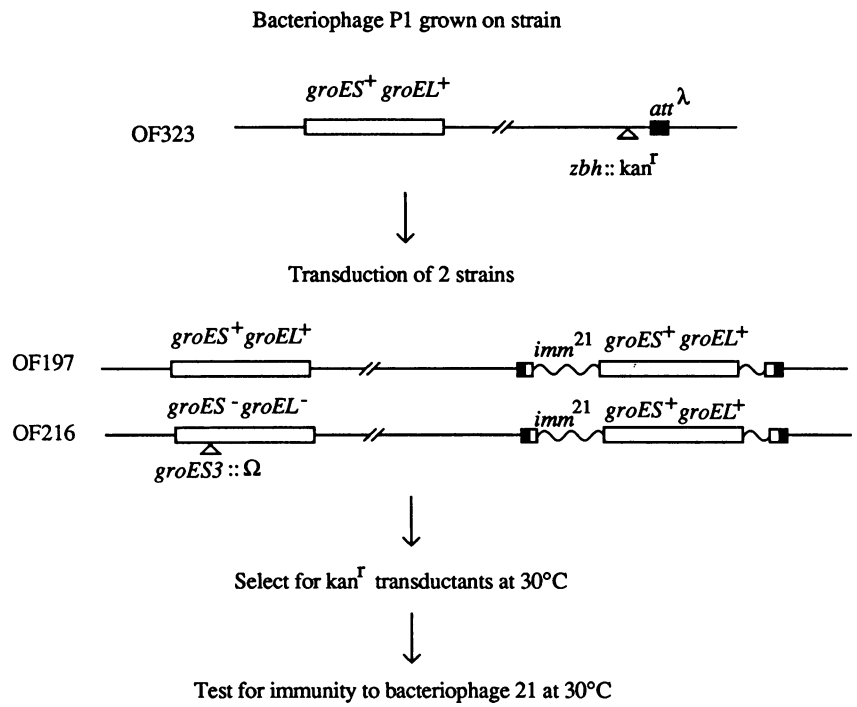
(stock preparations, lysogenization, and immunity tests) were carried out according to Arber et al. (1).

Molecular genetic procedures. The techniques used for plasmid construction (restriction endonuclease digestion, agarose gel electrophoresis, transformation, and plasmid purification) were carried out as described by Maniatis et al. (21).

Immunoblotting procedure. Proteins were separated by electrophoresis on 15% (wt/vol) polyacrylamide-sodium dodecyl sulfate gels and subsequently transferred to 0.2-μm-pore-size nitrocellulose filters (3). After transfer, the portion of the nitrocellulose filters that contained the molecular weight markers was stained with a 0.1% (wt/vol) amido black 10B solution in 25% (vol/vol) isopropanol–10% (vol/vol) acetic acid. The remaining portion was treated with 1% (wt/vol) bovine serum albumin to block unoccupied protein-binding sites, exposed to antisera prepared from rabbits that had been immunized with purified GroES or GroEL protein, and subsequently incubated with peroxidase-labeled goat anti-rabbit immunoglobulin G. Visualization of immunoactive bands was performed as described by Hawkes et al. (11).

RESULTS

The usual strategy to determine whether a gene is essential for cell viability is to try to replace the wild-type gene with a nonfunctional copy (14, 16, 17, 22). This can be attempted by transformation of a *recBC sbcB* or *recBC sbcA* mutant strain with a linear DNA fragment bearing an inactivated gene (14). Another approach that has been used is to cure cells for a λ transducing bacteriophage which contains the only functional copy of the gene of interest (16, 17, 22). As described below and illustrated in Fig. 1 and 2, we used a third method, also of general applicability (22, 38), based on bacteriophage P1-mediated transduction. Two protocols were used; for both, the essential step was construction of strains heterodiploid for the *groE* operon (OF216 and OF246; see Materials and Methods and Table 1). In these strains, one copy of the *groE* operon is functional and carried by a λ



RESULTS

Recipient Strain	Selected Marker	Scored Marker	
		λ*imm*^{21} *c*$^{-}$ *groES*$^{+}$*L*$^{+}$ sensitive	λ*imm*^{21}*c*$^{-}$ *groES*$^{+}$*L*$^{+}$ resistant
OF197	*zbh* :: kan^{r}	57	10
OF216	*zbh* :: kan^{r}	0	392

FIG. 1. Frequency of elimination by P1 transduction of the λ *imm*^{21} *cI*$^{+}$ *groES*$^{+}$ *groEL*$^{+}$ prophage from strains containing an additional wild-type or inactivated *groE* operon. The important genotypic features of the bacterial strains are indicated (see also Table 1). The Kan^{r} transductants were tested for each transduction by cross-streaking against bacteriophage λ *imm*^{21} *c groES*$^{+}$ *groEL*$^{+}$ at 30°C on L-agar plates containing 25 μg of kanamycin per ml.

groE^{+} bacteriophage (λ117; Table 1) inserted at the *att*λ site (17 min on the genetic map; 2). The other copy, located at its normal position on the chromosome (i.e., 94.2 min; 2) has been inactivated by a polar insertion of a DNA fragment (Ω fragment coding for resistance to both spectinomycin and streptomycin; 28) in the middle of *groES* coding sequence, the first gene of the *groE* operon (33). Since the *groES* and *groEL* genes are transcribed from promoters located in front of the *groES* gene (4, 39; K. Tilly, Ph.D. thesis, University of Utah, Salt Lake City), the Ω insertion should be extremely polar on the transcription of the *groEL* gene (28). In addition, strain OF246 contains a Tn*10* transposon (*zjd-1*::Tn*10*) inserted near the *groE* region (approximately 60% cotransfer between Tet^{r} and the *groE* genes by P1 transduction).

Requirement of one or both functional *groE* genes for growth. In the protocol described in Fig. 1, the heterodiploid strain OF216 (*groES*$^{+}$ *groEL*$^{+}$/*groES3*::Ω*groEL*) was used as a recipient in a bacteriophage P1 transduction. The aim of this experiment was elimination of the λ117 prophage carrying the wild-type *groES*$^{+}$ *groEL*$^{+}$ genes at the *att*λ site. Such an elimination should result in a cell possessing only the inactivated copies of the *groES* and *groEL* genes (*groES3*::Ω insertion). However, such transductants should be viable if and only if the *groES*$^{+}$ *groEL*$^{+}$ gene products are dispensable for *E. coli* growth. The donor strain (OF323) contains a mini-Kan^{r} derivative of Tn*10* (Table 1; 37) inserted near the *att*λ site (90% cotransduction by P1). After infection of OF216 with a bacteriophage P1 lysate prepared on the donor strain, Kan^{r} transductants were selected at 30°C. A homodiploid (*groES*$^{+}$ *groEL*$^{+}$/*groES*$^{+}$ *groEL*$^{+}$) recipient strain, OF197, was also included in the experiment as a control. The Kan^{r} transductants were subsequently tested at 30°C for the presence or loss of the immunity conferred by prophage λ117 (i.e., immunity to bacteriophage 21; Table 1). For the control homodiploid strain, 90% of the transductants should have lost immunity to bacteriophage 21. For the *groE* heterodiploid recipient, depending on whether the *groE* genes are dispensable or not, 90 or 0% of the Kan^{r} clones should have lost immunity to bacteriophage 21.

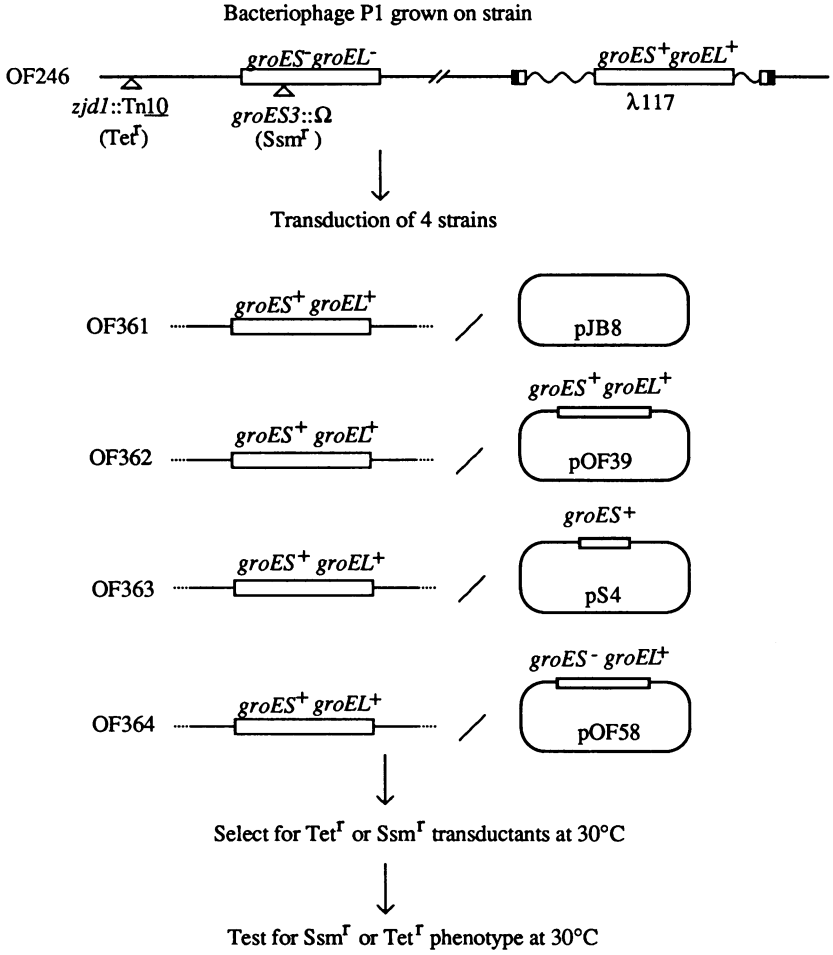
The results of the experiment are presented in the lower part of Fig. 1. After transduction of the control strain (OF197), the λ117 (λ *groES*$^+$ *groEL*$^+$) prophage was indeed recombined out at the expected frequency of 90%. For the heterodiploid, however, loss of the λ117 prophage did not occur at a detectable rate (0 of 392), as expected if either or both of the *groES*$^+$ and *groEL*$^+$ genes are essential or become essential when an unknown gene is inactivated by the *zbh-5*::Tn*10* insertion. To distinguish between these two possibilities, the experiment described above was repeated with another donor strain (OF377) carrying a mini-Tn*10* Kan^r insertion into the *gal* operon, which is 43% cotransductible with *att*λ (2). In that case also (data not shown), none of the OF216 Kan^r transductants had lost the λ117 prophage. We have repeated these transductions at 17°C and observed the same cotransduction frequencies, which indicated that, unlike *rpoH* (39), at least one of the *groE* gene products remained essential for *E. coli* growth at temperatures below 20°C.

Requirement of both *groES*$^+$ and *groEL*$^+$ for growth at 30°C. The aim of the protocol described in Fig. 2 was to determine whether it was possible to introduce the inactivated *groES* and *groEL* genes in strains containing an additional copy of either *groES*$^+$ or *groEL*$^+$. To do this, one of the two heterodiploid strains previously described (OF246; Table 1 and above) was used as the donor strain to transduce different plasmid-containing strains. The Tet^r transductants were selected at 30°C (i.e., transfer of *zjd-1*::Tn*10*) and then tested at 30°C for inheritance of the Ssm^r phenotype (transfer of the *groES3*::Ω insertion inactivating both the *groES* and *groEL* genes because of the extreme polarity of the Ω insertion; 28). For reasons discussed below, the Ssm^r transductants were selected in parallel. The recipient strains carry on their chromosomes the wild-type *groES*$^+$ *groEL*$^+$ operon. In addition, three of them possess, on a plasmid, either an extra copy of the entire *groES*$^+$ *groEL*$^+$ operon (OF362) or only one functional *groE* gene (*groES*$^+$ for OF363 and *groEL*$^+$ for OF364). The fourth strain, OF361, served as a negative control, since its plasmid does not carry any chromosomal genes. From the results of the previous set of transduction experiments (Fig. 1 and above), it was anticipated that transduction of this fourth control strain would not give rise to any Tet^r Ssm^r transductants. A positive transduction control should be provided by the strain carrying the *groES*$^+$ *groEL*$^+$ operon on a plasmid (OF362); in this case, 60% of the Tet^r transductants should also be Ssm^r. With the two remaining strains (OF363 and OF364), the frequency of Tet^r Ssm^r transductants will depend on whether one or both of the *groES* and *groEL* gene products are essential for bacterial growth. If only one of the gene products is essential, one transduction should result in approximately 60% Tet^r Ssm^r clones among Tet^r transductants. If both gene products are essential, no Ssm^r clones should be found among the Tet^r clones from either transduction. The results of this set of transductions are presented in the lower part of Fig. 2. In each case, a few hundred Tet^r transductants were tested for the Ssm^r phenotype. The negative and positive control strains (OF361 and OF362) gave the expected values, i.e., 0 and 60% of Ssm^r clones, respectively, among the Tet^r colonies. The two other strains gave Tet^r Ssm^r transductants but at a much lower frequencies than was found with the positive control strain (i.e., 2.7% for OF363 and 3.8% for OF364; Fig. 2). However, upon further examination, all of the rare transductants tested proved to be heterodiploids in which a wild-type *groE* operon coexisted with mutated forms (*groES3*::Ω insertion and deletions of plasmids pS4 and pOF58). In these rare, exceptional transductants, it was shown by Western blot (immunoblot) analysis that both the *groES*$^+$ and *groEL*$^+$ gene products were present (see below). Since the strains used are *recA*$^+$, it is not surprising that, in addition to the P1 particle-chromosome recombination events, plasmid-chromosome and plasmid-transducing particle exchanges may also occur, resulting in the formation of heterodiploids.

The rare Tet^r Ssm^r transductants of OF363 and OF364 were tested for the presence of the *groE* gene products by Western blot analysis followed by immunostaining. Extracts were prepared from the transductants and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteins were then transferred to nitrocellulose and immunostained by using polyclonal antibodies directed against either the GroES or the GroEL protein. Several classes of rare transductants were observed. Lane 1 of Fig. 3 shows a representative example of the Tet^r Ssm^r transductants of OF363. In all of these transductants, both the GroES and GroEL proteins were detected at levels similar to those seen in the OF361 control strain. Since the parental strain in this transduction overproduced the *groES* gene product, we conclude that a genetic rearrangement somehow resulted in loss of a functional *groES* gene from the plasmid. Analysis of the rare transductants of OF364 provided further evidence of genetic rearrangements. Based on the relative levels of the GroE proteins in these transductants, several classes could be defined (Fig. 3, lanes 2 through 5). Both GroE proteins were present in each class of transductant, which indicated that these rare Tet^r Ssm^r transductants did in fact carry a wild-type copy of the *groE* operon. In addition, the transductant represented in lane 3 was found, by DNA restriction analysis, to bear the Ssm^r insert on its plasmid (data not shown), which provided direct evidence for recombination between the plasmid and transducing DNA.

From these results, we conclude that an inactivated *groES-groEL* operon cannot be cotransduced with the *zjd-1*::Tn*10* insertion at a detectable frequency in strains carrying extra copies of only a wild-type *groES*$^+$ or a wild-type *groEL*$^+$ gene. This conclusion suggests that either (i) the *groES*$^+$ and *groEL*$^+$ gene products are essential for cell growth at 30°C or (ii) the *zjd-1*::Tn*10* insertion inactivates a gene that is essential only when one or both of the *groES* and *groEL* genes are nonfunctional. To address this second possibility, we carried out directed selection for transfer of the *groES3*::Ω inactivating insertion (Fig. 2). Such Ssm^r transductants should be obtained for the strain whose plasmid carries the wild-type operon (OF362), and about 60% of them should also have received the *zjd-1*::Tn*10* insertion. No transductants should be found in the case of the strain containing the pJB8 plasmid (OF361), since we know that at least one of the *groE* genes is essential (Fig. 1 and above). If both *groE* gene products are required for cell growth at 30°C, the last two strains (OF363 and OF364) should also yield no transductants. These predictions were fulfilled except in the case of strain OF364 (harboring the *groES*$^-$ *groEL*$^+$ plasmid [pOF58]), which yielded two Ssm^r transductants (Fig. 2). Once again, however, these rare Ssm^r resistant colonies proved to be heterodiploids retaining wild-type copies of the *groE* genes in addition to the inactivated gene (data not shown).

Taken together, results presented above strongly suggest that the presence of a functional copy of both the *groES* and *groEL* genes is required for bacterial growth at 30°C.



RESULTS

Recipient Strain	Selected Marker	Scored Marker		Selected Marker	Scored Marker	
		Ssmʳ	Ssmˢ		Tetʳ	Tetˢ
OF361	*zjd1*::Tn10	0	323	*groES3*::Ω	0	0
OF362	*zjd1*::Tn10	133	75	*groES3*:: Ω	96	64
OF363	*zjd1*::Tn10	8	294	*groES3*::Ω	0	0
OF364	*zjd1*::Tn10	9	234	*groES3*::Ω	2	32

FIG. 2. Frequency of bacteriophage P1-mediated transfer of inactivated *groE* genes into strains haploid or diploid for the *groE* operon. The important genotypic features of the bacterial strains are indicated (see also Table 1). Several hundred Tetʳ transductants were analyzed for each transduction by streaking on selective plates containing 40 μg of spectinomycin per ml. Conversely, the Ssmʳ transductants were streaked on selective plates supplemented with 20 μg of tetracycline per ml. All incubations were carried out at 30°C. The four recipient strains must be resistant to bacteriophage λ, because the P1 stock grown on strain OF246 contains spontaneously released λ imm^{21} *groE* phages. Some of these λ imm^{21} phages have acquired, by homologous recombination, the *groES3*::Ω insertion and can thus give rise at a relatively high frequency to spectinomycin-resistant colonies upon infection of a λ-sensitive strain.

DISCUSSION

The two bacteriophage P1 transduction protocols shown in Fig. 1 and 2 were designed to determine whether it was possible to construct strains in which no functional gene of interest (the *groES* and *groEL* genes, in this case) is present. To do this, we determined the cotransduction frequency between a selected marker (Tetʳ or Kanʳ, provided by transposon insertions) and an unselected marker (Ssmʳ, resulting from an insertional inactivation of the *groES* and *groEL* genes) or sensitivity to bacteriophage λ imm^{21} *c* $groES^+$ $groEL^+$ (resulting from loss of a λ imm^{21} $groES^+$ $groEL^+$ prophage). An essential step in this approach was the construction of strains heterodiploid for the gene of interest; i.e., one copy is wild type and the other is inactivated by the extremely polar insertion of an antibiotic resistance marker. Such a construction allows manipulation

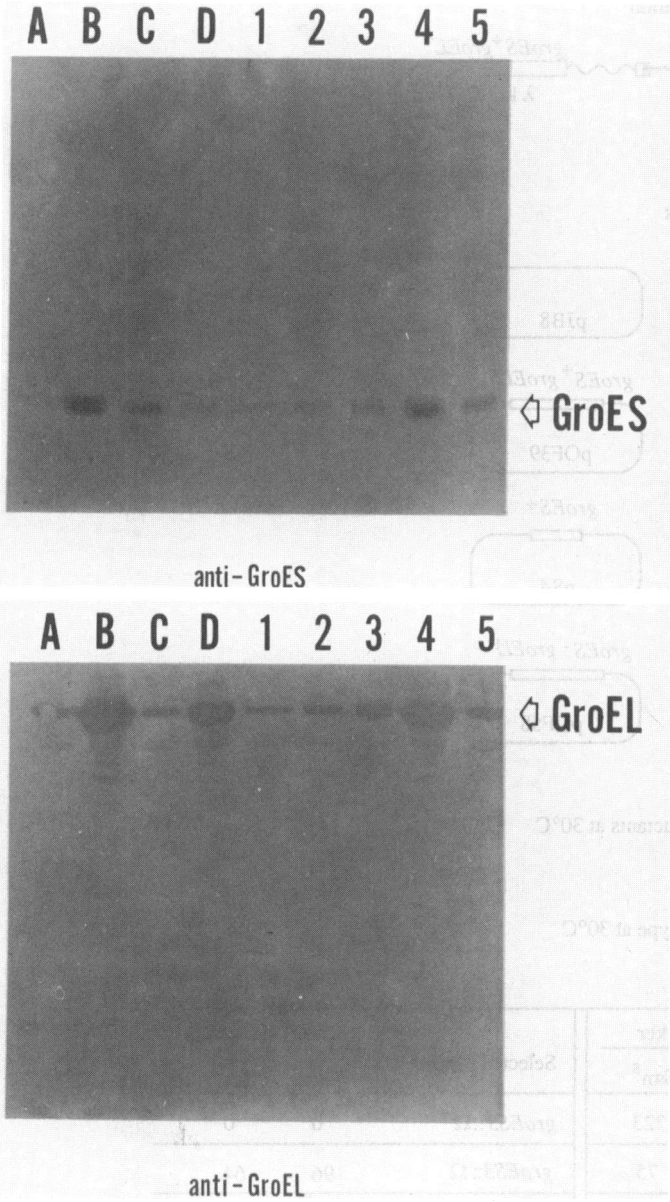


FIG. 3. Western blot analysis of extracts prepared from rare Tet^r Ssm^r transductants obtained with strains OF363 and OF364. Duplicate blots were immunostained by using either anti-GroES or anti-GroEL antibodies. Lanes: A, OF361; B, OF362; C, OF363; D, OF364; 1, representative of Tet^r Ssm^r transductants of OF363; 2 through 5, representatives of Tet^r Ssm^r transductants of OF364.

of the gene under study in vivo (P1 transduction, λ transducing bacteriophage, and suitable nearby transposon insertions) and in vitro (cloned fragment carrying the gene). Most of these requirements are also met by other approaches to the same problem (14, 16, 17, 22, 38). However, one advantage of the strategy that we used is that it can be carried out over a wide temperature range (from about 10 to 43°C). This ability was crucial, since we wanted to determine whether the *groES groEL* gene products are essential for growth at low temperature. A second advantage is that the recipient strains do not need to carry potentially interfering mutations such as *recBC sbcB* or *polA*(Ts) (14, 17).

Our transduction experiments prove that functional *groES* and *groEL* gene products, in addition to being essential at high temperature (7), are required for bacterial growth at lower temperatures (at least one of the *groE* gene products is required for growth even at 17°C). This finding suggests that the GroE proteins must be implicated in a fundamental cellular process. The nature of this process remains to be determined. Analysis of certain *groES*(Ts) or *groEL*(Ts) mutations revealed a pleiotropic phenotype; i.e., after transfer to high temperature, RNA, DNA, and protein syntheses were impaired (36). In addition, the Tr phenotype caused by the *groES131* mutation can be suppressed by an additional mutation in the *rpoA* gene, which codes for the α subunit of the RNA polymerase (35). The GroE proteins may therefore be necessary directly or indirectly for efficient macromolecular syntheses. The existence of a relationship between the *groE* genes and DNA replication is supported by the observation that amplification of these two genes can suppress temperature-sensitive mutations in a bacterial gene (*dnaA*) involved in the specificity of initiation of *E. coli* DNA replication (5, 15). In addition, it has been recently reported that a mutation in the *groEL* gene (*groEL411*) suppressed the Tr phenotype of bacteria carrying the *ssb-113* mutation (30). Interestingly, *ssb-113* was not suppressed by overproduction of the GroE proteins, which suggested that the *groEL411* mutation may allow the GroEL protein to assume a new function. It may be that this new function is the ability to assemble the *ssb-113* mutant protein into functional tetramers.

A morphogenetic role for the *groE* gene products is demonstrated at least in the case of bacteriophage λ. The GroES and GroEL proteins are required, together with λ gpNu3, for the formation of a dodecamer of λ gpB. The resulting preconnector structure (18) then initiates the correct assembly of the other components, leading to formation of a functional bacteriophage prohead (19).

The importance of the role of the *groE* genes in the heat shock response of *E. coli* is demonstrated by experiments with strains lacking the *rpoH* gene (encoding the RNA polymerase σ^{32} factor, which mediates expression from heat shock promoters; 39). When spontaneous temperature-resistant revertants of a Δ*rpoH* mutant were examined, it was found that all of them had acquired an IS*10* insertion upstream of the *groE* genes which resulted in a σ^{70}-dependent increase in the levels of *groE* gene products. These revertants were capable of growth at temperatures as high as 40°C, whereas the Δ*rpoH* parent is inviable at temperatures above 20°C (20). It has been shown that the *groE* operon possesses a σ^{70}-dependent promoter 25 to 30 bases downstream of the σ^{32}-dependent promoter. Furthermore, in a Δ*rpoH* strain, the σ^{70}-dependent *groE* promoter is actively transcribed (39), a finding which is in excellent agreement with our genetic analysis of the dispensability of the *groE* gene products at temperatures below 20°C.

Whatever the role of the GroE proteins, it appears that their function may have been well conserved through the course of evolution. Recently, it has been shown that the GroEL protein of *E. coli* is 46% identical (at the amino acid sequence level) to the Rubisco subunit-binding protein of plant chloroplasts (12). Furthermore, it appears that GroEL is also related to the *hsp58* gene product of eucaryotes, which has been shown to accumulate in mitochondria after heat shock (23, 24).

ACKNOWLEDGMENTS

This research was supported by Public Health Service grants to C.G. and by a Public Health Service training grant to T.Z., all from the National Institutes of Health.

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